



Effects of Dietary Garlic Powder on Growth, Feed Utilization and Whole Body Composition Changes in Fingerling Sterlet Sturgeon, *Acipenser ruthenus*

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ABSTRACT: A 12 week growth study was carried out to investigate the supplemental effects of dietary garlic powder (GP) on growth, feed utilization and whole body composition changes of fingerling sterlet sturgeon *Acipenser ruthenus* (averaging weight, 5.5 g). Following a 24-h fasting, 540 fish were randomly distributed to each of 18 tanks (30 fish/tank) under a semi-recirculation freshwater system. The GP of 0.5% (GP0.5), 1% (GP1), 1.5% (GP1.5), 2% (GP2) and 3% (GP3) was added to the control diet (GP0) containing 43% protein and 16% lipid. After the feeding trial, weight gain (WG) of fish fed GP1.5, GP2 and GP3 were significantly higher ($p < 0.05$) than those of fish fed GP0, GP0.5 and GP1. Feed efficiency and specific growth rate (SGR) showed a similar trend to WG. Protein efficiency ratio of fish fed GP1.5, GP2, and GP3 were significantly higher ($p < 0.05$) than those of fish groups fed the other diets. A significant difference ($p < 0.05$) was found in whole body composition (moisture, crude protein, crude lipid, ash, and fiber) of fish at the end of the experiment. Significantly higher ($p < 0.05$) protein and lipid retention efficiencies (PRE and LRE) were also found in GP1.5, GP2, and GP3 groups. Broken-line regression model analysis and second order polynomial regression model analysis relation on the basis of SGR and WG indicated that the dietary optimal GP level could be greater than 1.77% and 1.79%, but less than 2.95% and 3.18% in fingerling sterlet sturgeon. The present study suggested that dietary GP for fingerling sterlet sturgeon could positively affect growth performance and protein retention. (**Key Words:** *Acipenser ruthenus*, Garlic Powder, Protein Retention, Weight Gain, Feed Efficiency, Body Composition)

INTRODUCTION

Fish is a highly nutritive and rich source of animal proteins. For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide an artificial feed, by which fish grow rapidly and attain maximum weight in shortest possible time (Bhosale et al., 2010). One approach is to include new substances into fish diets to improve feed conversion efficiency or elevate general conditions for fish growth and maintenance (Fernández-Navarro et al., 2006). Plants are natural source of safer and cheaper chemicals. Beneficial

effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions (Citarasu, 2010).

Plant products have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation and immunostimulation in aquaculture practices (Citarasu et al., 2001, 2002; Sivaram et al., 2004). Garlic (*Allium sativum*) is a perennial bulb-forming plant that belongs to the genus *Allium* in the family *Liliaceae*. Garlic has been a subject of considerable interest for centuries as a flavouring agent, traditional medicine, and a functional food to enhance physical and mental health. Garlic was studied in different forms of extracts: aqueous, ethanol and dried powder (Shin and Kim, 2004). It contains a variety of organosulfur compounds such as allicin, ajoene, S-

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allylcysteine, diallyl disulfide, S-methylcysteine sulfoxide and S-allylcysteine (Chi et al., 1982). A wide array of beneficial effects of garlic such as antihypertensive, antihyperlipidemic, antimicrobial, hypoglycaemic, anticancer, antidote (for heavy metal poisoning), anticarcinogenic, hepatoprotective and immunomodulation have been reported by several researchers (Foushee et al., 1982; Macmahon and Vargus 1993; Agarwal, 1996; Agusti, 1996; Bordia et al., 1996; Yeh and Liu, 2001). Studies on garlic as an alternative growth promoter in livestock production were conducted and its beneficial effects on growth, digestibility and carcass traits have been reported (Bampids et al., 2005; Tataru et al., 2008). Dietary garlic as a growth promoter in Nile tilapia (*Oreochromis niloticus*) improved body weight gain (WG), feed intake and feed efficiency (FE) (Diab et al., 2002; Shalaby et al., 2006).

In Korea, sturgeon aquaculture began in 1996 with the main species of sterlet sturgeon (*Acipenser ruthenus*), Siberian (*A. baeri*), Russian (*A. gueldenstaedti*), Stellate (*A. stellatus*) and hybrid called bester (beluga female×sterlet male). The sterlet is freshwater species which very rarely ascends the brackish water of rivers (Krayushina, 2006).

Our previous research suggested dietary garlic for juvenile sterlet sturgeon (60 to 100 g) could positively affect growth performance (Lee et al., 2012), but no trial was conducted to study the effect of dietary garlic powder (GP) for fingerling sterlet sturgeon to date. Therefore, this

study was designed to investigate the effects of garlic powder on growth performance and whole body composition changes of the fish.

MATERIALS AND METHODS

Experimental design and diets

Fingerling sterlet sturgeons (*Acipenser ruthenus*) were obtained from Gyeonggi Province Maritime and Fisheries Research Institute, Gyeonggido, Korea. This experiment was designed to determine the optimum levels of GP as a growth promoter and carried out to investigate the supplemental effect of dietary GP on whole body composition changes of fingerling sterlet sturgeon during 12 weeks. Six groups (three replicates/group) of 540 fish of a mean body weight of 5.5 g selected from 2,500 fish were randomly allotted to each of 18 tanks (2×1×0.4 m). Fish were cultured in semi-recirculation freshwater system. Water temperature and dissolved oxygen levels were kept at 21±1°C and over 6 mg O₂/L, respectively. Flow rate was adjusted at a minimum level of 10 L/min.

Composition and proximate analysis of the experiment diets are shown Table 1. The GP of 0.5%, 1%, 1.5%, 2%, and 3% each was added to the basal diet (GP0) containing 43% protein and 16% lipid and the diets were designated as GP0.5, GP1, GP1.5, GP2, and GP3. Experimental diets were manufactured at the size of 1.5 mm by a twin extruder

Table 1. Ingredient composition and chemical analysis of the experimental diets¹

Ingredients	GP0	GP0.5	GP1	GP1.5	GP2	GP3
LT fish meal	20	20	20	20	20	20
Fish meal	15	15	15	15	15	15
Wheat flour	20	20	20	20	20	20
Corn gluten meal	0	0	0	0	0	0
Soybean meal	22	22	22	22	22	22
Squid liver powder	2	2	2	2	2	2
Lecithin	1	1	1	1	1	1
Choline	0.2	0.2	0.2	0.2	0.2	0.2
Betaine	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin mix	0.1	0.1	0.1	0.1	0.1	0.1
Mineral mix	0.1	0.1	0.1	0.1	0.1	0.1
Fish oil	9.4	9.4	9.4	9.4	9.4	9.4
Filler as rice bran	10	9.5	9	8.5	8	7
Garlic powder ²	0	0.5	1	1.5	2	3
Chemical composition (% DM) ³						
Protein	43.8	43.7	43.5	42.4	43.2	42.8
Lipid	15.5	15.5	15.6	16.1	16.0	16.1
Ash	9.7	9.8	9.8	9.4	9.3	9.7
Fiber	6.5	6.4	5.9	6.1	6.0	5.4
Gross energy (kJ/g)	22.6	22.7	21.4	21.3	21.2	21.0

GP, garlic powder; LT, low temperature; DM, dry matter.

¹ All ingredients were provided with Cargill Agri Purina Ltd., Korea.

² Obtained from local market at Gyeonggi Province, in Korea.

³ Values are means of three determinations.

(Model ATX-2, Fesco Precision Co., Daegu, Korea) in Shinjang-Bios Company in Korea. Diets were fed by hand at the rate of 4% to 5% of fish body weight per day at 08:00, 13:00, and 18:00 for 12 weeks. All diets were stored at -20°C prior to use.

Sample collection and analysis

At the end of the feeding trial, fish were anesthetized with AQUI-S (New Zealand Ltd., Lower Hutt, NZ) and then weighed as total fish weight of each replicate within groups and counted for calculation of WG, FE, specific growth rate (SGR), protein efficiency ratio (PER) and survival rate. Six fish (two fish per tank) from each group were selected at similar weight to determine hepatosomatic index (HSI) and condition factor (CF). Fifteen fish (five fish per tank) from each group were selected to analyze whole-body proximate composition. Chemical analyses of diets and fish whole body were performed by the standard procedure of AOAC (1995) for moisture, crude protein and crude ash. Crude lipid was determined using the Soxtec system 1046 (Tecator AB, Hoganas, Sweden) after freeze-drying the samples for 12 hours. Crude fiber was measured as loss on ignition of dried lipid free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH (FOSS instrument, Hoganas, Sweden) and gross energy was determined using an automatic adiabatic bomb calorimeter (PARR instrument, USA).

Statistical analysis

Data (WG, FE, SGR, PER, HSI, CF, whole body compositions and retention efficiency) were analyzed using one-way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan's multiple range test (Duncan, 1955). Broken-line

analysis (Robbins et al., 1979) was used to estimate the optimum dietary GP levels and the second-order polynomial regression analysis (Zeitoun et al., 1976) was introduced to the optimum dietary GP requirement of fingerling sterlet sturgeon. Significance was tested at 5% level and all statistical analyses were carried out using the SPSS Version 10 (SPSS, Michigan Avenue, Chicago, IL, USA).

RESULTS

Growth performance

At the end of the 12 wk feeding, WG, FE, SGR, PER, HSI, and CF and survival of fingerling sterlet sturgeon fed the experimental diets were determined and shown in Table 2. Fish fed GP1, GP1.5, GP2, and GP3 exhibited significant improvement in WG, FE, SGR, PER, and CF as compared to those of fish fed the control diet GP0 ($p < 0.05$). Final weight of fish was the highest in fish fed GP3 (30.2 g), while the lowest (25.6 g) was found in fish fed GP0. The CF (0.52) of fish fed GP2 was significantly higher ($p < 0.05$) than that of fish fed diet, it was, however, not significantly different from that of fish fed GP3 ($p > 0.05$). The highest HSI (2.08%) was found in fish fed GP0 among treatments ($p < 0.05$). Although fish fed GP3 showed the lowest HSI of 1.71% among fish groups, it was not significantly different ($p > 0.05$) from three other groups (GP1, GP 1.5, and GP2). No mortality was recorded in all fish groups during the experimental period. Based on the on-way ANOVA, broken-line regression model analysis and second order polynomial regression model analysis relation on SGR and WG, the optimum dietary p level (%) could be greater than 1.77% and 1.79% (Figures 1 and 2), but less than 2.95% and 3.18% in fingerling sterlet sturgeon under our experimental conditions (Figures 3 and 4), respectively.

Table 2. Growth performance of fingerling sterlet sturgeon fed diets without (GP0) or with (GP0.5, GP1, GP1.5, GP2 and GP3) garlic powder diets for 12 wk¹

Growth performance	Diets						p-value
	GP0	GP0.5	GP1	GP1.5	GP2	GP3	
Initial average weight (g)	5.50±0.03	5.46±0.02	5.51±0.05	5.48±0.02	5.53±0.03	5.51±0.02	
Final average weight (g)	25.64±1.12	26.14±0.28	27.01±0.39	29.60±0.25	30.16±0.20	30.18±0.21	
WG (%) ²	366.4±2.3 ^c	379.2±4.3 ^b	390.1±5.9 ^b	440.4±6.2 ^a	445.0±3.7 ^a	448.0±3.1 ^a	<0.001
FE (%) ³	69.3±3.7 ^c	70.9±0.5 ^{bc}	73.2±1.2 ^b	82.0±1.3 ^a	84.0±0.9 ^a	84.2±0.4 ^a	<0.001
SGR (%) ⁴	1.83±0.04 ^c	1.87±0.01 ^{bc}	1.89±0.01 ^b	2.01±0.01 ^a	2.02±0.01 ^a	2.02±0.01 ^a	0.001
PER ⁵	1.62±0.09 ^b	1.62±0.01 ^b	1.68±0.03 ^b	1.93±0.03 ^a	1.94±0.02 ^a	1.97±0.01 ^a	0.003
HSI (%) ⁶	2.08±0.13 ^a	1.87±0.05 ^b	1.81±0.04 ^{bc}	1.83±0.08 ^{bc}	1.74±0.09 ^c	1.71±0.11 ^c	0.001
CF ⁷	0.36±0.04 ^c	0.43±0.03 ^{bc}	0.42±0.04 ^{bc}	0.42±0.06 ^{bc}	0.52±0.08 ^a	0.44±0.07 ^{ab}	0.001
Survival rate (%)	100	100	100	100	100	100	

GP, garlic powder; WG, weight gain; FE, feed efficiency; SGR, specific growth rate; PER, protein efficiency ratio; HSI, hepatosomatic index; CF, condition factor; SE, standard error; DM, dry matter.

¹ Means of three replicates groups, values are presented as mean±SE. Means in each row with different superscripts are significantly different ($p < 0.05$).

² WG (%) = [final weight (g) - initial weight (g)] × 100 / initial weight (g). ³ FE (%) = wet weight gain (g) × 100 / feed intake (g, DM).

⁴ SGR (%) = {ln final weight (g) - ln initial weight (g)} / days × 100. ⁵ PER = wet weight gain (g) / protein intake (g, DM).

⁶ HSI (%) = liver weight (g) × 100 / body weight (g). ⁷ CF = [fish weight (g) / fish length (cm)³] × 100.

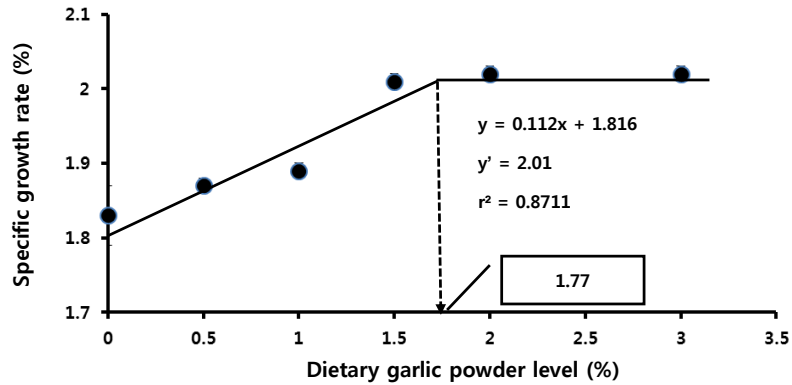


Figure 1. Broken-line analysis on specific growth rate of fingerling sterlet sturgeon to dietary garlic powder levels.

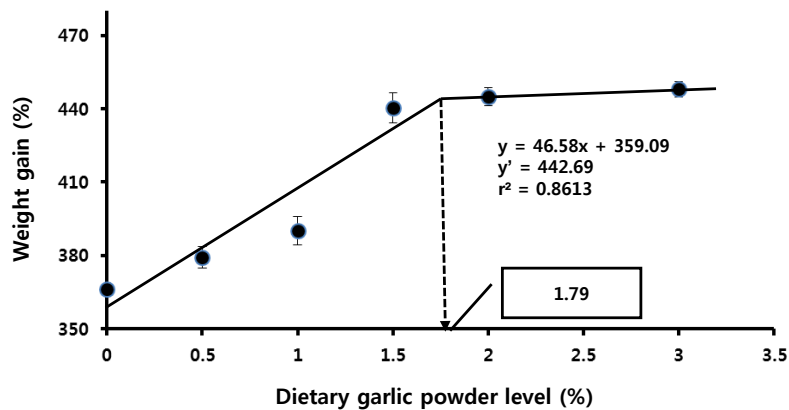


Figure 2. Broken-line analysis on weight gain of fingerling sterlet sturgeon to dietary garlic powder levels.

Whole body compositions

The whole body proximate composition of initial and final fingerling sturgeon fed diets without (GP0) or with (GP0.5, GP1, GP1.5, GP2, and GP3) GP during 12 weeks is shown in Table 3. Moisture ranged from 75.1% (GP1.5) to 77.5% (GP0.5) among fish groups, while that of initial fish was 84.9%. Crude protein and lipid ranged from 13.1% (GP0) to 15.1% (GP1.5) and from 5.6% (GP3) to 6.7% (GP0), respectively. Fish fed GP3 showed the highest ash

content (3.3%), while that of fish fed GP0.5 showed the lowest value (2.5%). Fiber content ranged from 1.2% (GP1) to 2.2% (GP1.5). The proximate composition was significantly different ($p < 0.05$) among the six experimental fish groups. Protein retention efficiency (PRE) varied from 22.9% (GP0.5) to 31.2% (GP1.5) and lipid retention efficiency (LRE) from 33.2% (GP1) to 39.1% (GP0), which were also significantly different ($p < 0.05$) among fish groups.

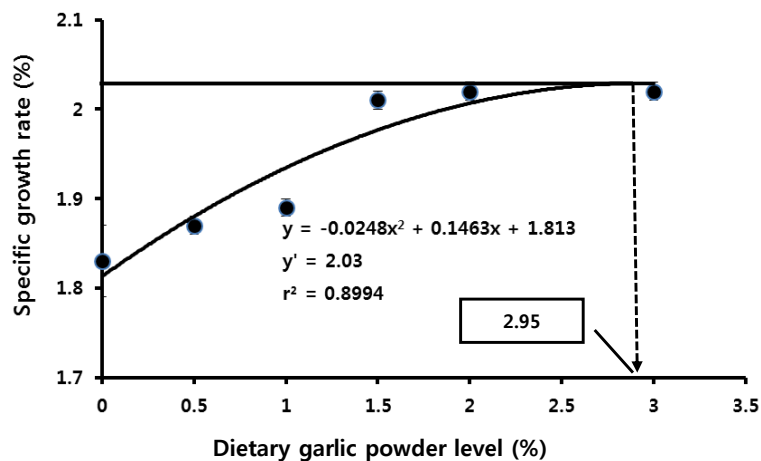


Figure 3. The second order polynomial analysis on specific growth rate of fingerling sterlet sturgeon to dietary garlic powder levels.

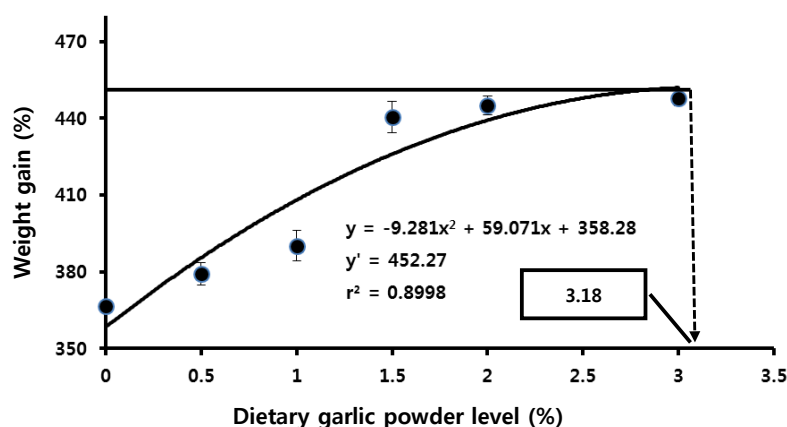


Figure 4. The second order polynomial analysis on weight gain of fingerling sterlet sturgeon to dietary garlic powder levels.

DISCUSSION

Hung et al. (1998) carried out a growth trial for white sturgeon having 72 g body weight. They fed fish one of seven commercial salmonid diets or a purified diet for 8 wk. These diets contained 5.9% to 8.3% moisture, 31.5% to 51.4% crude protein, 7.6% to 19.4% lipid and 3.8% to 11.7% ash. The best growth and FE was obtained from a diet with 4.5% moisture, 51.4% crude protein, 18.0% lipid and 11.7% ash. However, none of the detailed data was reported on the optimal levels of the remaining nutritive components in the feeds for fingerling sterlet sturgeon (over 5.5 g). Lim (2009) reported that the optimum dietary protein requirement level for fingerling sterlet sturgeon (7 to 40 g) could be above 44.3% with the optimum dietary protein to energy ratio of 21.9 mg protein/kJ diet. The graded levels of GP were incorporated into the diet GP0 to investigate dietary protein sparing and the addition of GP showed a significant improvement in WG, FE, SGR, and PER.

Several herbs such as garlic, onion, marjoram, caraway, basil, anise, fennel, licorice, black seed and fenugreek have been tested for growth promoting activities (Jayaprakas and

Eupharsia, 1996; Citarasu et al., 2002; Sivaram et al., 2004), feed conversion (Shalaby et al., 2003; El-Darkar et al., 2004a,b; Shalaby, 2004), and improvement of protein digestibility and energy retention (El-Dakar et al., 2004a,b) in aquatic animals. Results in Table 2 are in agreement with that reported by Soltan and El-Laithy (2008) who reported that the incorporation of garlic in diets for growing Nile tilapia significantly improved WG and SGR. Shalaby et al. (2006) also reported a significant increase in WG, FE, PER, and SGR of Nile tilapia fed diet containing 3% GP. Similarly, Diab et al. (2002) mentioned feeding diet with 2.5% garlic resulted in the highest growth performance in *O. niloticus*. In the same species, Abou-Zeid (2002) found a positive improvement in biomass and SGR with garlic supplementation. Metwally (2009) also mentioned that the best performance was obtained in Nile tilapia fed diet with 3.2% GP. A significant increase in growth, feed conversion and protein efficiency was shown in rainbow trout when fed diet with 1.0% garlic (Nya and Austin, 2009). Abdel-Hakim et al. (2010) reported that incorporation of garlic into Nile tilapia diets (diet with fresh garlic 3 g per kg) resulted in significant improvement in WG, feed conversion, protein efficiency and CF. Protein efficiency ratio and FE are

Table 3. Proximate composition of whole body of fingerling sterlet sturgeon fed without (GP0) or with (GP0.5, GP1, GP2, and GP3) garlic powder diets for 12 wk (%; as-is basis)¹

Treatments	Moisture	Crude protein	Crude lipid	Ash	Fiber	PRE ²	LRE ³
Initial	84.90±0.32	10.22±0.09	1.19±0.05	2.42±0.02	0.18±0.01		
GP0	77.30±0.05 ^a	13.06±0.17 ^d	6.71±0.07 ^a	3.01±0.09 ^b	2.05±0.08 ^b	23.2±0.4 ^c	39.1±0.5 ^a
GP0.5	77.47±0.27 ^a	13.32±0.11 ^d	5.99±0.05 ^b	2.45±0.06 ^c	1.75±0.07 ^c	22.9±0.2 ^c	33.4±0.2 ^d
GP1	76.88±0.25 ^{ab}	13.99±0.03 ^c	6.05±0.02 ^b	2.87±0.08 ^b	1.19±0.06 ^d	24.3±1.2 ^b	33.2±1.4 ^d
GP1.5	75.11±0.48 ^c	15.06±0.44 ^a	5.84±0.09 ^c	3.24±0.10 ^a	2.21±0.04 ^a	31.2±0.5 ^a	34.1±0.5 ^{cd}
GP2	75.50±0.01 ^c	14.82±0.07 ^a	5.62±0.14 ^d	2.93±0.15 ^b	2.04±0.06 ^b	30.8±0.3 ^a	34.7±0.3 ^c
GP3	76.29±0.64 ^b	14.49±0.11 ^b	5.59±0.05 ^d	3.27±0.01 ^a	1.27±0.04 ^d	30.4±0.1 ^a	36.7±0.1 ^b
p-value	0.003	<0.001	0.001	0.002	<0.001	<0.001	0.001

GP, garlic powder; PRE, Protein retention efficiency; LRE, lipid retention efficiency; SE, standard error.

¹ Values are means±SE of three replicates with 2 determinations per replicate for each final group. Means with different superscripts in the same row are significantly different (p<0.05). ns, non significant (p>0.05).

² PRE (%) = body protein gain (g)/dry protein intake (g)×100. ³ LRE (%) = body lipid gain (g)/dry lipid intake (g)×100.

utilized as quality indicators for fish diets and amino acid balance. Therefore, these factors are used to evaluate protein utilization and turnover (Shalaby et al., 2006). The present results are also in agreement with those obtained by Khattab et al. (2004) who found that the diet of Biogen increased feed intake, improved feed conversion ratio (FCR) and PER in tilapia. In contrast to these results, however, Sahu et al. (2007) reported that SGR and FCR in fish (*Labeo rohita*) fed diet with 0.5% and 1% GP was not significantly different from those of the control. In livestock, a few studies suggested that garlic did not affect growth performance (Horton et al., 1991; Freitas et al., 2001; Bampidis et al., 2005) because of the pungent smell which may lead to lower diet palatability. Jegede (2012) reported that apparent protein digestibility was significantly improved from 71.2% for control diet to 85.2% for a diet with 2% garlic. Khalil et al. (2001) mentioned that garlic contains allicin, which promotes the performance of the intestinal flora, thereby improving digestion and enhancing the utilization of energy, leading to improved growth. Wanapat et al. (2008) found significantly higher digestibility, absorption and retention of N in GP supplemented groups compared to control group. Those results also accord with our present finding that sturgeons fed GP1.5, GP2.0 and GP3.0 had higher N utilization (PRE, %) than other groups. From these results, it was concluded that the addition of GP of 1.79% to 3.18% was optimal for growth performance of fingerling sterlet sturgeon and revealed that addition of GP could spare dietary protein in terms of growth and feed utilization, although protein digestibility was not determined.

The HSI provides an indication of the status of energy reserve in an animal. As the liver is a target for the metabolism in the fish body, the liver index (HSI) is a useful biomarker to detect the hazardous effects of environmental factors (Pait and Nelson, 2003). The present results showed that fish groups fed GP containing diets had significantly lower HSI than that of fish group fed GP0. Our results are in agreement with the finding of Metwally (2009) who reported that diets fed for three months containing different forms of garlic, natural garlic (40 g per kg diet), garlic oil capsules (250 mg per kg diet) significantly decreased HSI of fingerling Nile tilapia. In contrast, Shalaby et al. (2006) showed that incorporation of garlic into Nile tilapia diets (10, 20, 30, and 40 g per kg diet) had no significant effects on HSI. Al-Salahy and Mahmoud (2003) reported that garlic caused significant hypolipidaemia in response to repeated doses, accompanied by a decrease in liver total lipids and a significant rise in white muscle total lipids. In the present study, HSI was lower in fish groups fed diets containing higher GP (GP2 and GP3) than that of the group fed GP0 which showed the lowest growth performance. Therefore, such observed

hepatomegaly in the GP0 group may partially reflect that HSI level deviates from the normal range in fingerling sterlet sturgeon because of high dietary lipid and that GP administration (GP diets) may lead to a significant decrease in liver lipid.

Dietary lipids are important nutrient affecting energy production and essential for growth and development in most of fish. But, fish are known to utilize protein preferentially to lipid or carbohydrate as an energy source. In our previous study (Lee et al., 2012), lipid content in whole body of juvenile sterlet sturgeon fed diet with 0.5% garlic extract was significantly higher (6.1%) than that (5.4%) of fish fed control. However, in the present study the higher dietary GP significantly decreased body lipid from 6.71% (GP0) to 5.59% (GP3). In addition, when compared to our previous study (Lee et al., 2012), PRE was higher and LRE (%) was lower in all of six experimental fish groups. The reason might be due to the difference in dietary garlic utilization between garlic extract and GP and to the difference in fish size between previous (65 to 250 g) and present (5 to 30 g) studies. As well, it might be suggested that fingerling sterlet sturgeon utilize protein more than lipid in their diet.

In conclusion, broken-line regression model analysis and second order polynomial regression model analysis relation on the basis of SGR and WG indicated that the dietary optimal GP level could be greater than 1.77% and 1.79%, but less than 2.95% and 3.18% in fingerling sterlet sturgeon. The present results suggest that dietary garlic for fingerling sterlet sturgeon (5.5 to 30.0 g) could positively affect growth performance and feed utilization.

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